Ultrastructural Abnormalities in the Skeletal Muscle of Children with Chronic Cholestasis and a Long-Term Vitamin E Replacement

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KOBAYASHI, Y., TAZAWA, Y., NAKAGAWA, M., HIGASHI, O. and YAMAMOTO, T.Y. Ultrastructural Abnormalities in the Skeletal Muscle of Children with Chronic Cholestasis and a Long-Term Vitamin E Replacement. Tohoku J. exp. Med., 1988, 154 (3), 285-296 — We investigated the effects of long-term oral and intramuscular vitamin E repletion in children with chronic cholestasis. Clinical improvement or suppression of neuromuscular involvement after adequate vitamin E repletion was demonstrated. Light and electron microscopic abnormalities of the skeletal muscle, however, did not completely disappear despite the correction of the biochemical abnormalities for more than 3 years. The muscle fibers showed less variety of pathologic features than before vitamin E treatment. Inclusions observed in the skeletal muscle fibers before vitamin E treatment were still observed in subsarcolemmal cytoplasm and the perinuclear regions. They were more irregularly curved and consisted of various substances. Similar inclusions were also observed in Schwann cells, perineural cells, fibroblasts, pericytes, endothelial cells and smooth muscle cells of intramuscular vessels. Although the external lamina was not disrupted, separation of the external lamina from the plasma membrane and multilayered external lamina were often observed. The nerves among muscle fibers still showed degenerative features. Morphological changes of the skeletal muscle during vitamin E therapy have not so far been reported in cases of chronic cholestasis. We discuss the relationship of these findings to vitamin E replacement in children with chronic cholestasis.

vitamin E; cholestasis; muscle pathology; ultrastructure

The relationship between prolonged vitamin E deficiency and neurologic complications in patients with cholestasis has been well documented (Rosenblum et al. 1981; Guggenheim et al. 1982a, b, 1983; Alvarez et al. 1983; Neville et al.

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Chylomicron formation and an adequate intraluminal concentration of bile salts are the most important factors for efficient absorption of vitamin E (Muller et al. 1974). Sokol et al. (1983a) revealed that markedly impaired biliary secretion during severe cholestatic liver disease leads to concentrations of intraluminal bile acids below the critical micellar concentration. The resulting impaired mixed micelle formation causes vitamin E malabsorption and subsequent vitamin E deficiency. Administration of oral vitamin E in massive doses does not usually normalize serum concentrations of vitamin E in these patients unless given together with conjugated bile acid (Sokol et al. 1983a). Since there are obvious practical limitations and theoretical disadvantages in giving children large doses of bile acids orally, several groups have administered vitamin E by the intramuscular route to children with chronic cholestasis (Guggenheim et al. 1982a, b; Muller et al. 1983; Alvarez et al. 1985; Sokol et al. 1985; Perlmutter et al. 1987).

We previously reported ultrastructural abnormalities in the skeletal muscle of a patient with severe cholestasis, vitamin E deficiency and neurologic involvements (Kobayashi et al. 1984). In the present study, we investigate the effects of long-term oral and intramuscular vitamin E repletion on neurologic function in children with chronic cholestasis and demonstrate that light microscopic and ultrastructural abnormalities of the skeletal muscle still remained after 3 years of vitamin E repletion, although biochemical abnormalities had disappeared.

**Subjects and Methods**

Case history of Patient 1 (Y.Y.), a 7-year-old girl, was previously reported (Kobayashi et al. 1984). Patient 2 (T.Y.), her younger brother, was born to nonconsanguineous parents with no history of hepatic diseases. Persistent jaundice and severe itching had been noticed since early infancy. At the age of 11 months, he was admitted to Tohoku University Hospital because of persistent jaundice, intensive pruritus and recurrent diarrhea. A paucity of the intrahepatic bile ducts was proven by liver biopsy. Neurologic complications in Patient 2 (T.Y.) before vitamin E therapy was less severe than in Patient 1 (Y.Y.) (Table 1). On admission both patients had low levels of serum vitamin E, vitamin E/total lipid ratios and severe cholestasis with serum bile acid levels of more than 250 \( \mu \text{mol/liter} \) (Table 2).

Serum vitamin E was measured by a fluorometric assay (Thompson et al. 1973). Vitamin E deficiency was defined as a serum level less than 0.5 mg/100 ml or a ratio of serum vitamin E level to serum total lipid level less than 0.6 mg/g (Farrell et al. 1978). Serum bile acids were analyzed by an enzymatic method (Mashige et al. 1976) and gas-liquid chromatography (Kimura et al. 1979; Tazawa and Konno 1982).

Oral administration of high doses of \( \alpha \)-tocopherol acetate (50–100 mg/kg/day) normalized the serum vitamin E level. However, after cholestyramine therapy for jaundice and pruritus, serum vitamin E levels and vitamin E/total lipid ratios decreased below the critical level despite continuous oral vitamin E supplementation (Nakagawa et al. 1984). During cholestyramine treatment, serum total bile acid levels transiently increased about twofold in both patients (Nakagawa et al. 1984). Intramuscular administrations of vitamin E (50–100 mg/week) were required to achieve normal serum vitamin E levels and vitamin
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E/total lipid ratios in both patients (Table 2). During the treatment, neurological complications were improved or suppressed by sufficient vitamin E therapy (Table 1).

Muscle biopsies were performed in only Patient 1 (Y.Y.) before vitamin E therapy and in both patients after 3 years of sufficient vitamin E therapy. Specimens of the quadriceps femoris muscle (before vitamin E therapy) or the biceps brachii muscle (after 3 years of vitamin E therapy) were fixed in 5% glutaraldehyde and 4% paraformaldehyde or 2.5% glutaraldehyde in cacodylate buffer, pH 7.3, and postfixed in 1% osmium tetroxide. They were dehydrated in graded concentration of ethanol and embedded in epoxy resin. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined with an electron microscope (JEM-100C, JEOL, Tokyo). Thick sections were stained with toluidine blue for light microscopy.

**RESULTS**

We previously reported the light and electron microscopic findings of the muscle biopsy in Patient 1 (Y.Y.) before vitamin E therapy (Kobayashi et al. 1984).

**Light microscopic findings (after vitamin E repletion)**

Small granule-like deposits were observed in a few muscle fibers. The muscle fibers showed less variety of pathologic features than before vitamin E repletion. Three typed of abnormalities of muscle fibers were observed before vitamin E therapy: (1) In the first type abnormality, thin and atrophic muscle fibers and
widened endomysium were noted. (2) The second type of abnormal muscle fibers contained abundant vacuoles, occasionally arranged in a whirled pattern and stained irregularly dark. (3) As the third type of abnormality, a few homogenous muscle fibers with large nuclei containing a prominent nucleolus were scattered among intact muscle fibers (Kobayashi et al. 1984).

Even after 3 years of sufficient vitamin E repletion, a few first and third type abnormal muscle fibers were still found in both patients. The endomysium among muscle fibers contained an increased number of intramuscular capillaries (Fig. 1). The connective tissue and mononuclear cells in the endomysium were less than those before vitamin E therapy.

**Electron microscopic findings (after vitamin E repletion)**

The inclusion bodies were observed in the skeletal muscle fibers of Patient 1 (Y.Y.) before vitamin E treatment. They were located in intermyofibrillar cytoplasm, subsarcolemmal cytoplasm and the perinuclear regions (Kobayashi et al. 1984). Similar inclusions were still observed in subsarcolemmal cytoplasm and the perinuclear regions of the skeletal muscle fibers in both Patients 1 and 2 even after 3 years of vitamin E repletion. They were more irregularly curved and consisted of various substances; homogenous, finely granular and granular of high osmiophilic densities (Fig. 3). Elongated inclusion bodies with a crystallloid structure (Fig. 2) and large polymorphous bodies with aggregations of membrane or myelin figures (Fig. 4) were often observed, especially in Patient 1 (Y.Y.). Similar inclusions were also observed in Schwann cells, perineural cells, fibroblasts, pericytes, endothelial cells and smooth muscle cells of intramuscular vessels.

Attenuated myofibrils were often noted in the first type of abnormal muscle fibers in both Patients 1 and 2 (Fig. 5). Accumulations of large polymorphous mitochondria with obvious cristae and vacuoles in subsarcolemmal cytoplasm (Fig. 7), and Z-band streaming were also observed in these muscle fibers (Fig. 6).
Few severely degenerated muscle fibers were noted among them after vitamin E repletion. There were neither disruption nor disappearance of the sarcolemma. Although the external lamina was not disrupted, separation of the external lamina from the plasma membrane and multilayered external laminae were often observed (Fig. 3). Most intramuscular capillaries possessed multilayered of thickened external laminae. Empty external laminae were often observed in the endomysium (Fig. 8). A few peripheral nerves among muscle fibers showed abnormal features characterized by focal vacuolization or a thin myelin sheath (Figs. 9 and 10).

Pathologic findings found in this study were less variable and severe, compared with those before vitamin E supplement. In Patient 2 (T.Y.), pathologic findings were similar to those of Patient 1 (Y.Y.), but not identical in severity. Generally, morphologic abnormalities in Patient 2 (T.Y.) were milder in severity than those in his elder sibling.

**DISCUSSION**

Clinical improvement or suppression of neuromuscular involvement after adequate vitamin E repletion has been demonstrated in our patients. Morphological abnormalities, however, did not completely disappeared despite the correction of the biochemical abnormalities for more than 3 years. Morphological changes of the skeletal muscle during vitamin E therapy were documented in two cases of abetalipoproteinemia by Azizi et al. (1978) and Lazaro et al. (1986), but not in cases of chronic cholestasis. Repeated muscle biopsies in Azizi's case showed apparent improvement in histologic abnormalities, but Lazaro's case worsened despite vitamin E therapy and apparent preservation of strength. Vitamin E treatment in the latter case of abetalipoproteinemia (Lazaro et al. 1986) and our cases of chronic cholestasis might have produced normal serum vitamin E concentrations and vitamin E/total lipid ratios, but incomplete repletion of tissue vitamin E stores. This may explain why children have an increased
Fig. 1. Light micrograph of the skeletal muscle fibers showing slightly atrophic muscle fibers, homogenous muscle fibers (arrow) and the endomysium with an increased number of intramuscular capillaries. Toluidine blue stain. ×170.

Fig. 2. Electron micrograph of an elongated inclusion body with a crystalloid structure in subsarcolemmal cytoplasm. ×13,000.

Fig. 3. A large inclusion body consisted of various substances and multilayered external laminae (*). ×16,800.
Fig. 4. Large polymorphous bodies with aggregations of membrane or myelin figures. ×19,000.

Fig. 5. Thin attenuated myofibrils in the first type of abnormal muscle fibers. ×11,300.

Fig. 6. Z-band streaming in the muscle fiber. ×8,400.
susceptibility to vitamin E deficiency and no resistance to depletion of endogenous vitamin E storage depots. Morphological abnormalities may be closely related to the vitamin E store in the tissue. The serum vitamin E/total lipid ratio is thought indirectly to indicate a vitamin E level in the tissue but not a vitamin E store. There is no marker of tissue vitamin E stores.

We previously suggested that degenerative and regenerative processes occurred repeatedly in a vitamin E deficient status (Kobayashi et al. 1984). Multilayered or thickened basal laminae of capillaries and empty basement membrane of the skeletal muscle fibers were frequently found after vitamin E replacement, which suggests that degenerative and regenerative processes occurred repeatedly during vitamin E therapy and indicates a persistent, subclinical vitamin E deficiency in the tissue. Autophagic vacuoles containing nondescript debris and myelin figures were often observed in the skeletal muscle fiber after vitamin E therapy. They may possibly contain the same or a similar substance in different stages of evolution or gradation of dense inclusion bodies. They are also considered to be some forms of residual bodies indicating mild vitamin E deficiency in the tissue.

Corwin (1980) has recently summarized various experiments of mitochondrial function in vitamin E deficient animals and concluded that vitamin E probably played a role in the early part of the electron-transport system near the dehy-
Fig. 8. Empty external laminae observed in the endomysium (arrow). ×19,500.
Fig. 9. Peripheral nerves among muscle fibers showing focal vacuolization of the myelin sheath. ×9,500.
Fig. 10. Peripheral nerves among muscle fibers showing a thin myelin sheath (arrow). ×7,800.
drogenase steps. Alphatocopherol, structurally similar to coenzyme Q, is considered to be the most important moiety because it is the most abundant and exibits the greatest biological activity (Cohn 1975). Abnormal mitochondria of skeletal muscles in our cases were still found after vitamin E therapy, which indicates that tissue was still in vitamin E deficiency.

Age-related differences in response to intramuscular vitamin E therapy were reported recently by Sokol et al. (1985), with greater responses in younger and less severely affected patients. They also showed that complete reversal or prevention of clinical neurologic abnormalities was possibly obtained when the vitamin E repletion was started before the age of 3 years. Unfortunately, they did not investigated morphological aspects of the neuromuscular system after vitamin E therapy. We initiated vitamin E administration at ages of 3 years in Patient 1 (Y.Y.) and 11 months in Patient 2 (T.Y.). Ultrastructural neuromuscular abnormalities of the skeletal muscle in Patient 2 (T.Y.) still remained after 3 years adequate vitamin E repletion, although the abnormalities were less marked than in Patient 1 (Y.Y.).

Incomplete improvements in morphologic findings of the skeletal muscle in our patients may be related to the longstanding, severe cholestasis before the treatment, resulting in an extreme depletion of vitamin E store in the tissue. Sokol et al. (1983b) recommended that aggressive attempts to correct vitamin E deficiency should be initiated early in life. However, the absolute requirement for vitamin E for normal maintenance of the nervous system and the skeletal muscle has not been elucidated. Vitamin E-deficient neuromuscular disease often takes years to develop, and an equally long time is probably needed to achieve its clinical improvement and much longer for morphological reverse once effective supplements have been initiated.

It is also possible that factors other than vitamin E deficiency contribute to neuromuscular disease in chronic cholestatic liver disease. Several reports (Sokol et al. 1983b; Wichman et al. 1985; Lazaro et al. 1986; Perlmutter et al. 1987) suggested that the myopathy in vitamin E deficiency might be due to some other mechanisms and not to vitamin E deficiency per se. In chronic cholestasis, interactions between vitamin E and other exogenous and endogenous prooxidants and antioxidants (e.g. selenium-glutathione peroxidase, copper, iron and other nutritional factors) may limit the protective function of vitamin E, thereby accelerating lipoperoxidation tissue injury. Further investigation will be necessary to determine whether or not other factors contribute to the development of neuromuscular involvement and its response to the replacement therapy.

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References


